

FIG. 1. CpG dinucleotide frequency in the hNIS promoter region. The DNA sequence of the hNIS promoter and its contiguous transcribed region (up to the first intron), was assessed by computer analysis. Nucleotide positions are in reference to the adenosine residue of the ATG translation start site. The bold arrow indicates the position of a 'TATA' box-like element. The shaded box (P) denotes the region of the hNIS promoter chosen for methylation analysis. The open box (L) and the solid box (C) denote the leader and coding regions, respectively, of the first exon which were analyzed for methylation status.

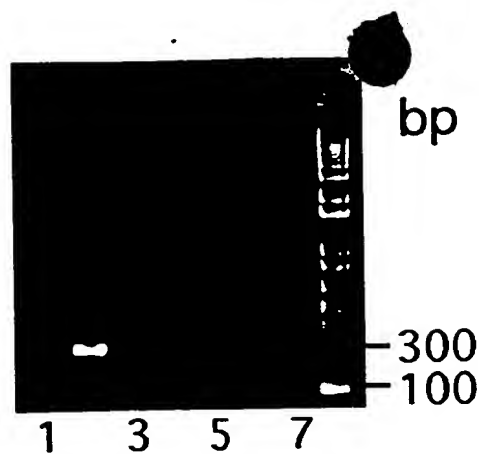


FIG. 2. hNIS mRNA expression in tall-cell papillary thyroid carcinoma. RT-PCR products were resolved on a 2% agarose gel and visualized by ethidium bromide staining. PCR substrates are: Lane 1, no cDNA (negative control); lane 2, normal thyroid (positive control); lanes 3-7, tall-cell papillary thyroid carcinomas (samples 11-15, Table 1); and lane 8, GIBCO-BRL 1 kb-plus DNA ladder.

09606042-062900

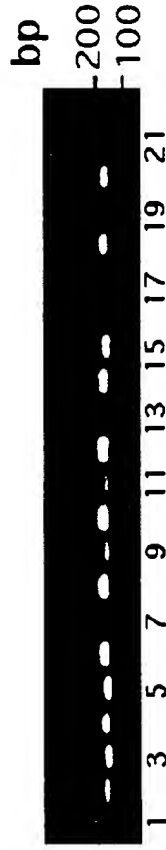
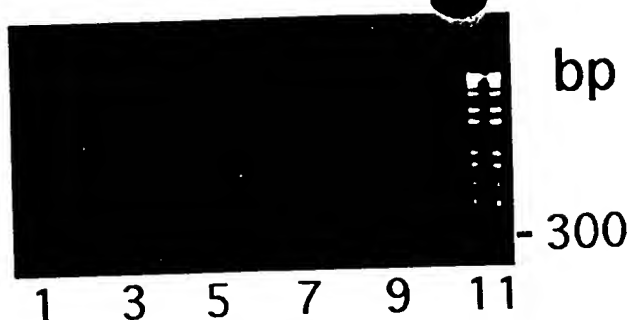


FIG. 3. Methylation analysis of the hNIS promoter in proximity to TATA box (Region P). Products of methylation specific-PCR analysis of sodium bisulfite modified genomic DNA from thyroid tumors using a methylation-specific primer pair (MET) and non-methylated-specific primer pair (UNMET) were electrophoresed on an agarose gel in adjacent lanes. Lanes 1 and 22: GIBCO-BRL 1 kb plus DNA ladder; lanes 2 through 21 (even numbered lanes contain the 151 bp UNMET product and odd numbered lanes contain the 143 bp MET product). Lane pairs starting with 2 to 12 represent the reaction pairs of tall cell papillary cancer samples 11 through 16, respectively (Table 1). Lane pairs starting with 14 through 20 represent the reaction pairs for anaplastic carcinoma (Table 1, Sample 22), negative control (no template DNA), normal thyroid and pooled human leukocyte DNA, respectively.



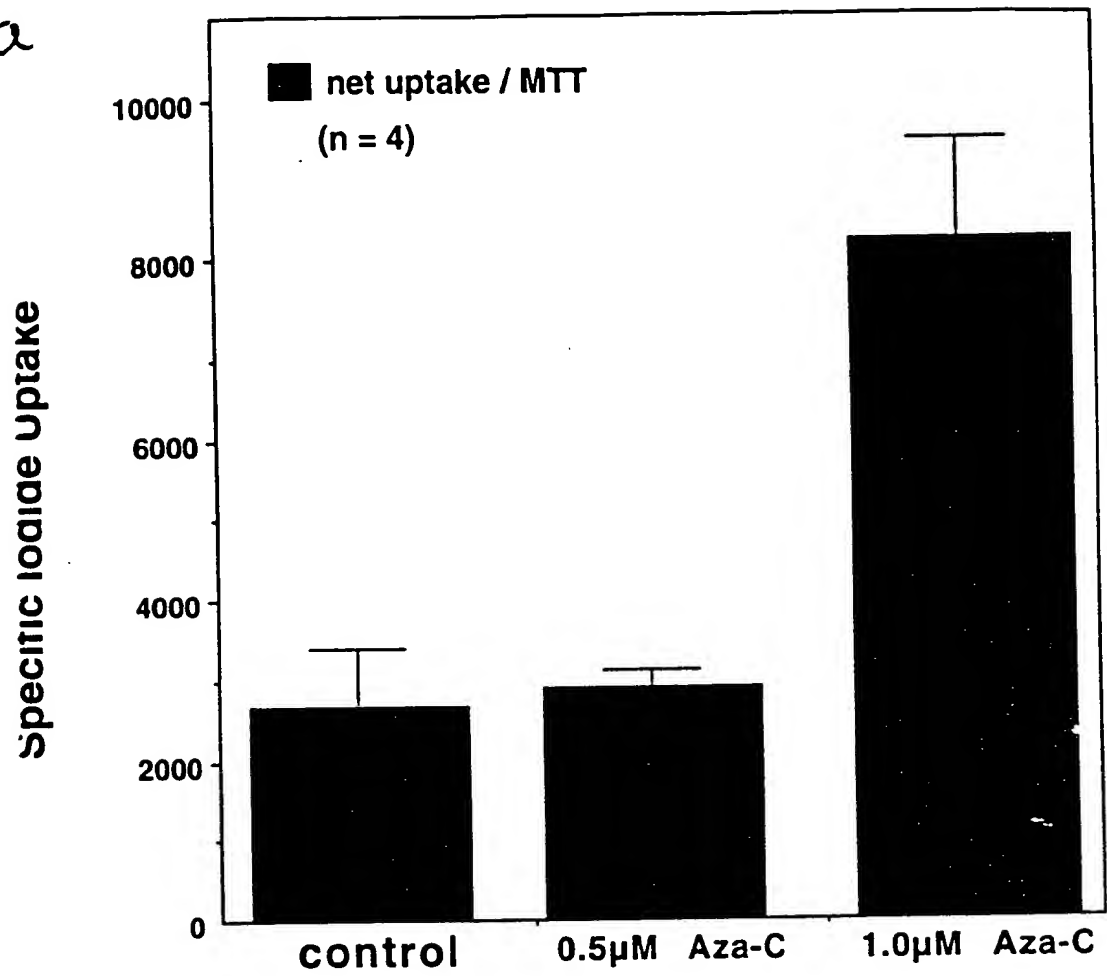
4a



4b

FIG. 4. Re-expression of hNIS mRNA in thyroid cell lines. Follicular adenoma cell line, KAK1 (a). KAK-1 cells were treated in triplicates, with 5-azacytidine as described. The RT-PCR products were resolved on a 2% agarose gel and visualized by ethidium bromide staining. lane1. no cDNA; lane 2 to 4. untreated; lanes 5 to 7. 0.5 μ M 5-azacytidine for 3 days (added each day); lanes 8 to 10. 1.0 μ M 5-azacytidine for 3 days (added each day); lane 11. GIBCO-BRL 1 kb plus DNA ladder. Papillary carcinoma cell line, NPA'87 (b). NPA'87 cells were treated in triplicates, with sodium butyrate or 5-azacytidine as described. The RT-PCR products were resolved on a 2% agarose gel and visualized by ethidium bromide staining. lane1. GIBCO-BRL 1 kb plus DNA ladder; lane 2. normal human thyroid; lane 3 to 5. untreated; lanes 6 to 8. 1.0 mM sodium butyrate for 3 days; lanes 9 to 11. 1.0 μ M 5-azacytidine for 3 days (added each day).

Fig 5
a

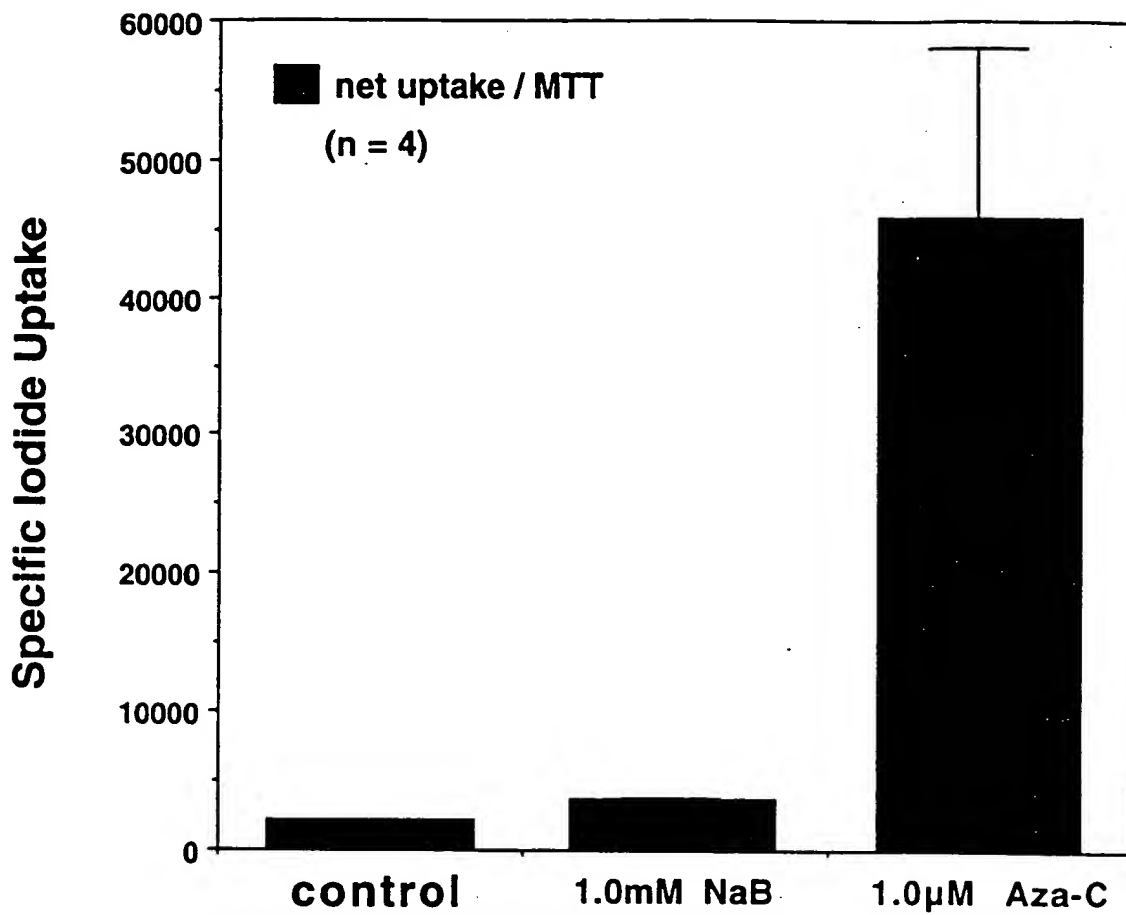


Restoration of iodide uptake in KAK-1 Cells

006290" 24090960

Fig. 5

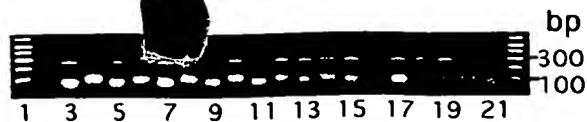
b



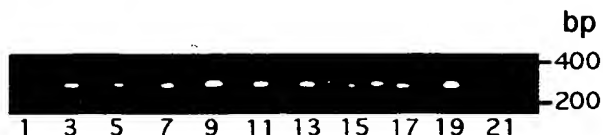
Restoration of iodide uptake in NPA '87 Cells



6a



6b



6c



6d

FIG. 6. Methylation analysis of hNIS gene regions in cell lines re-expressing hNIS mRNA. Products of methylation specific-PCR analysis of sodium bisulfite modified genomic DNA, from thyroid cell lines, using two methylation-specific primer pairs (MET for regions L and C) and two corresponding non-methylated-specific primer pair (UNMET for regions L and C) were electrophoresed on an agarose gel in adjacent lanes. In all gels, lanes 1 and 22: GIBCO-BRL 1 kb plus DNA ladder; lanes 2 through 7: triplicate pairs of cell lines under basal conditions; lanes 8 through 19: triplicate pairs of cell lines in two different treatment conditions; lanes 20 and 21: negative controls without template DNA (all even numbered lanes contain the respective UNMET products and odd numbered lanes contain the corresponding MET products). Cell line KAK-1 studied with primer pairs specific for region L (a). Treatment conditions in lanes 8-13 and lanes 14-19 include 5-azacytidine at 0.5 μ M and 1.0 μ M, respectively. Cell line KAK-1 studied with primer pairs specific for region C (b), with conditions identical to (a). Cell line NPA'87 studied with primer pairs specific for region L (c). Treatment conditions in lanes 8-13 and lanes 14-19 include sodium butyrate at 1.0 mM and 5-azacytidine at 1.0 μ M, respectively. Cell line NPA'87 studied with primer pairs specific for region C (d), with conditions identical to (c).

006290-2499960